CHAPTER 10

Establishing a Cell Line

Most investigators who use cell culture in their work will obtain their cell lines from other investigators or from a cell bank such as the American Type Culture Collection. It is certainly easier to obtain and use a previously established cell line than to create one. However, there may be instances in which no acceptable line exists with the desired properties, or is derived from the cell type or genetic background of interest. Transgenic and knockout animals also provide the opportunity to establish cell lines with known genetic anomalies. In these cases the investigators may wish to establish their own line in their laboratory. This chapter will deal with the techniques used to establish and characterize new cell lines from normal and transformed tissues.

TRANSFORMED CELL LINES

As previously mentioned, the best way to establish a cell line *in vitro* is to start with cells that are rapidly dividing *in vivo*. A tumor, by definition, is made up of cells that are rapidly dividing and will continue to do so, since they have escaped the normal growth control mechanisms of the cell. One can also purposely transform cells either *in vivo,* in order to obtain tumors that can be used to establish cell lines, or *in vitro.* Animal strains that have a high incidence of spontaneous tumors might be used to establish cell lines from these spontaneous tumors. Irradiation or chemical carcinogens can be used to induce tumors in animals. Alternatively, transformation may be induced *in vitro* by introducing transforming viruses or viral genes to primary cultures. A transforming gene with a regulable promoter (Hofmann *et al.,* 1992) may also be used to produce cell lines that can be switched from the transformed to normal phenotype.

More recently, transgenic animals have been produced that widely express transforming genes such as the SV40 T antigen. Tissues from these animals can be used to more easily establish cell lines *in vitro* (Noble *et al.,* 1995). Other transgenic animals have been created with targeted expression of transforming genes that predictably form tumors in specific tissues or cell types (Siegel *et al.,* 1994) or with gene deletions that predictably lead to tumor formation in specific tissues (Matzuk *et al.,* 1992). The main issues involved in ob-

taining cell lines from transformed cells is one of isolation of the cell type of interest and prevention of fibroblast overgrowth *in vitro* if these cultures are established in serum-containing medium. The approaches outlined above have led to the development of a number of cell lines derived from specialized cell types of exceptional interest. These lines are extremely valuable. It should be emphasized, however, that these cell lines are all, by definition, transformed and will therefore have properties significantly different from those of the normal tissue from which they are derived. These differences will of course frequently involve changes in the the growth regulation of these cells types.

TUMOR TISSUE

If a tumor is available and the goal is to establish a cell line from the tumor, the first step is to prepare a primary culture from the tumor tissue as described in Chapter 9 on primary culture. We prefer to use a serum-free medium, if possible, since this precludes any problems with fibroblast overgrowth and minimizes chromosomal instability and loss of some functional characteristics, as described in Chapter 8. If the cell type from which the tumor is derived has previously been grown in serum-free culture (e.g., primary culture or tumors from another species), then the previously used supplements make a good starting point. If the attempt to grow cells in serum-free medium fails, the addition of various amounts of serum may be tried starting with a small amount (e.g., 0.1%) and working up to the 10–20% level. This is best done in the presence of a "best guess" hormone supplement such as insulin and transferrin and trace elements. Several media may also be tried. Once the best conditions are determined for maximal survival in primary culture, the attempt to obtain a cell line can be made.

The main issues to keep in mind when trying to establish a cell line are:

1. Keep the cells in conditions that optimize their chance to grow (e.g., fresh medium, subconfluent cell density, periodic subculture, maximized medium nutrients and hormones).
2. Keep the cells at as high a density as is compatible with step 1 above.
3. Freeze cells down periodically during the process of trying to establish a cell line to insure against loss of the line owing to an accident and to allow for return to an earlier passage if desired properties are lost during the establishment of the cell line.

TRANSFORMING NORMAL CELLS *IN VITRO*

An alternative approach to obtaining transformed cell lines is to transform primary cultures of normal cells *in vitro*. This approach may be required when working with human tissues that do not grow indefinitely *in vitro* without some level of overt transformation. This approach also allows more control of the type of transforming agent and the extent of transformation than may be possible using cell lines derived from tumors. For example, one might conditionally transform cells using a temperature-sensitive SV40 T antigen or other controllable transforming agent.

To transform by viral or oncogene transfection of cells, one needs to choose the transforming gene to be used, the method of introducing the foreign DNA into the cells (if DNA is to be used), and the selection method to be used to select for the desired transformed phenotype. This has been covered extensively in other methods books (see Goeddel, 1991).